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Familial Spread of a Virulent Clone of *Klebsiella pneumoniae* Causing Primary Liver Abscess[▽]

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Capsule-forming *Klebsiella pneumoniae* K1 caused primary liver abscess in two household members of a family. The causative isolates had identical pulsed-field gel electrophoresis patterns and were determined to be sequence type 23. An additional member of the family was found to carry the same strain without clinical manifestation.

CASE REPORTS

Case 1. In January 2006, a 50-year-old male without underlying significant illness was diagnosed with liver abscess and treated empirically with fosfomycin for 3 weeks without drainage of the abscess and without documentation of the causative organism. Although the abscess disappeared once on computed tomography (CT) scans, it recurred in May 2007. The patient was admitted to Tokyo Teishin Hospital and drainage of the liver abscess was performed. Culture of the blood and aspirated pus yielded hypermucoviscous *Klebsiella pneumoniae* as the sole organism. The BacT/Alert 3D system (Sysmex bioMérieux, Tokyo, Japan) was used for the blood culture, and identification of the bacterial species was performed with the MicroScan WalkAway 40 SI system (Siemens Healthcare Diagnostics, Tokyo, Japan). Treatment with intravenous panipenem-betamipron, a carbapenem antibiotic available in Japan, for 4 weeks and subsequent treatment with oral ciprofloxacin for 4 weeks was successful. He had no history of travel around Asia except for Korea, which he had visited in 2000.

Case 2. In December 2009, the son of the patient in case 1, a 21-year-old previously healthy male, presented to Tokyo Teishin Hospital with a fever of 40°C and abdominal pain. He was hypotensive upon presentation, and the white blood cell count was 14,000/μl (reference range, 3,900 to 9,800), with 76% segmented cells and 13% bands. The values of serum aspartate aminotransferase, alanine aminotransferase, and creatinine were 94 U/liter (10 to 40), 138 U/liter (5 to 40), and 1.9 mg/dl (0.61 to 1.04), respectively. CT scans of the abdomen demonstrated a mass lesion measuring 7 cm in diameter and located in the right lobe of the liver. Serology results were negative for *Entamoeba histolytica*. After blood cultures were obtained and needle aspiration of the suspected liver abscess was carried out, empirical antibiotic therapy with imipenem-cilastatin was started. Blood and aspirated pus culture revealed hypermucoviscous *K. pneumoniae* as the sole organism. The antibiotic treatment was changed to pazufloxacin, a fluoroquinolone with a spectrum of activity similar to that of ciprofloxacin, and metronidazole for the coverage of anaerobic bacteria that may have been possibly involved in the infection. The patient soon became afebrile and clinically stable, and results of laboratory tests returned to normal. The antibiotics were administered intravenously for 3 weeks, and therapy with oral levofloxacin was continued for another 3 weeks. The symptoms resolved completely, and the follow-up CT scans showed no signs of recurrence of liver abscess. He had no complaints of visual disturbance or neurological symptoms during the course of the illness. He had no history of travel in Asia outside Japan.

The family members living with the patients, i.e., the wife and the daughter of the patient in case 1, had no significant medical and travel histories. In January 2010, stool and throat samples from the patient in case 1 and those of his wife and his daughter were cultured on bromothymol blue-lactose agar, and characteristic hypermucoviscous colonies of *K. pneumoniae* were selected from the colonies grown on the agar. A colony with formation of viscous strings >5 mm stretched with a standard inoculation loop was regarded as hypermucoviscous according to the criteria employed in the previous study (6). Stool and throat cultures of the wife of the patient in case 1 were positive for hypermucoviscous *K. pneumoniae*, whereas cultures of the samples obtained from other family members were negative for the pathogen. The *K. pneumoniae* isolates from the blood culture (TUM9518) and from aspirated pus (TUM9519) of the patient in case 2, a stored isolate from a blood culture of the patient in case 1 (TUM9520), and isolates from the stool (TUM9521) and throat (TUM9522) cultures of the wife of the patient in case 1 were available for subsequent analysis (Table 1).

The identification of the bacterial species of the isolates was reevaluated with an API 50 CHE system (Sysmex bioMérieux), which can differentiate *K. pneumoniae*, *Raoultella planticola*,

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TABLE 1. Characteristics of the bacterial strains analyzed in this study[a]

Parameter	Result for indicated isolate				
	TUM9518	TUM9519	TUM9520	TUM9521	TUM9522
Isolate source	Son	Son	Index patient	Wife	Wife
Sample	Blood	Pus	Blood	Stool	Throat
Time of isolation	Dec 2009	Dec 2009	May 2007	Jan 2010	Jan 2010
Growth at 10°C	−	−	−	−	−
Capsular serotype/genotype	K1	K1	K1	K1	K1
PFGE type	A	A	A	A	A
ST type	23	23	23	23	23
Indole test	−	−	−	−	−
Ornithine decarboxylase test	−	−	−	−	−
Voges-Proskauer test	+	+	+	+	+
O-Nitrophenyl-β-D-galactopyranoside test	+	+	+	+	+
Fermentation of:					
D-Arabinose	−	−	−	−	−
Dulcitol	+	+	+	+	+
L-Fucose	+	+	+	+	+
D-Melezitose	−	−	−	−	−
L-Sorbose	−	−	−	−	−
D-Tagatose	+	+	+	+	+

[a] The persons from whom the isolates were recovered are described according to relationship to the patient in case 1 (index patient). Jan, January; Dec, December.

and *Raoultella terrigena*, and growth at 10°C. The 5 isolates showed identical biochemical reactions and were determined to be *K. pneumoniae* with 99.9% probability by API 50 CHE system (Table 1).

Clonal relatedness of the isolates was assessed by pulsed-field gel electrophoresis (PFGE) of XbaI-restricted total DNA and multilocus sequence typing (MLST) performed according to the protocol described on the *K. pneumoniae* MLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) (5). All isolates showed identical banding patterns by PFGE, and MLST revealed that the isolates were of the sequence type 23 (ST23) strain (Table 1). Susceptibility testing of the isolates was performed with the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (4). The 5 isolates were susceptible to all the antibiotics tested, as follows: amoxicillin-clavulanate, ceftazidime, cefotaxime, cefepime, cefoxitin, imipenem, gentamicin, and ciprofloxacin. The capsular serotype was determined with *Klebsiella* antisera Seiken (Denka Seiken, Tokyo, Japan). Capsular polysaccharide synthesis genotyping PCR analysis to amplify the K serotype-specific alleles at *wzy* and *wzx* loci located in the capsular polysaccharide synthesis gene cluster was carried out as described by Fang et al. (7). The structure of the capsular polysaccharide synthesis gene cluster of *K. pneumoniae* is well conserved, and the alleles of *wzy* and *wzx* encoding integral inner membrane proteins are specific to each capsular genotype (7). The isolates were demonstrated to be capsular serotype/genotype K1 (Table 1). The presence of the virulence genes *rmpA*, *allS*, *mrkD*, *kfu*, *cf29a*, *fimH*, *uge*, *wabG*, and *ureA* was screened by PCR analysis by using relevant primers (1). The isolates were found to possess all the genes explored except for *cf29a*.

Recently, a novel form of *K. pneumoniae* infection was reported, which typically presents as community-acquired liver abscess with sepsis, sometimes complicated by metastatic endophthalmitis or central nervous system infections (7). While this clinical syndrome has mainly been reported from Taiwan, an increase in the cases of this syndrome has been described in Korea (3) and sporadic cases have been documented in other regions, including North America (9, 11) and Japan (10). Although a high prevalence of diabetes mellitus has been reported in patients who acquire this infection, it may also affect otherwise healthy individuals (7). The virulence gene *magA* located on the operon for capsular serotype K1 of *K. pneumoniae* has been identified as being responsible for the hypermucoviscous phenotype and resistance to host defense mechanisms in the causative organisms (2, 6). There is a high prevalence of K1 strains among clinical isolates of *K. pneumoniae* in Taiwan; this corresponds with the fact that this clinical syndrome is common in the region (8). Recent genetic analysis of *K. pneumoniae* isolates of diverse environmental and clinical origins collected worldwide demonstrated that isolates belonging to clonal complex 23 (CC23) (a group of clones having sequence type 23 [ST23] and closely related STs) were strongly associated with primary liver abscess among K1 strains and possessed several virulence genes, such as *allS* and *mrkD*, which were absent in other K1 isolates (1). Furthermore, in a previous study in Korea, all of the 73 isolated K1 strains causing liver abscess belonged to the CC23 group (3).

We demonstrated the clonal spread of a *K. pneumoniae* ST23 strain among three members of a family, which caused primary liver abscess in two of them. The causative isolates were susceptible to all the antibiotics tested, consistent with the findings in past studies of *K. pneumoniae* K1 isolates causing primary liver abscess in Taiwan (7). The isolates had the ability to use dulcitol and D-tagatose as carbon sources, which was demonstrated to be characteristic of CC23 K1 strains in a previous study (1). The patients in cases 1 and 2 were affected with liver abscess despite having no underlying immunosuppressive conditions or obstruction of the biliary tract. Although

samples from the wife of the patient in case 1 were positive for the same virulent *K. pneumoniae* isolate, she did not develop liver abscess. A retrospective cohort study involving patients with *K. pneumoniae* liver abscess showed that patients infected with K1 strains were more likely to be male (7), but it remains to be elucidated whether difference in gender is associated with the intensity of host defense against invasive *K. pneumoniae* K1 infection.

To the best of our knowledge, this is the first report of familial spread of *K. pneumoniae* K1 isolates causing liver abscess. The exact match of the PFGE pattern suggests that the familial spread of the K1 strain occurring through household contact or consumption of common foodstuffs was more likely than independent acquisition of the isolates. Although nobody in this family had visited Taiwan, where the virulent clone of *K. pneumoniae* K1 is widespread, travel to Korea might relate to the acquisition of the causative organism by the patient in case 1. Though the definite route of transmission was not clear, it is noteworthy that the same virulent clone of *K. pneumoniae* had been maintained among the family members for at least 2 years. The cases we described here suggest that transmission through household contact could occur with virulent *K. pneumoniae*, potentially leading to insidious dissemination of the organism in areas where it is not endemic.

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